LIVER DYSFUNCTION IN SEVERE BIRTH ASPHYXIA

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C E R T I F I C A T E

It is certified that the work in connection with thesis of Dr. Ram Prakash Gupta on "LIVER DYSFUNCTION IN SEVERE BIRTH ASPHYXIA" for M.D. (Paediatrics) Examination of Bundelkhand University, was conducted in the department of Paediatrics, M.L.B. Medical College, Jhansi.

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C E R T I F I C A T E

This is to certify that the work on "LIVER DYSFUNCTION IN SEVERE BIRTH ASPHYXIA", which is being submitted as a thesis for M.D.(Paediatrics) Examination of Bundelkhand University, has been carried out by Dr. Ram Prakash Gupta under my direct guidance and supervision in the department of Paediatrics. The techniques embodied in the thesis were undertaken by the candidate himself and observations recorded have been periodically checked by me.

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(Ram Prakash Gupta)

C O N T E N T

I N T R O D U C T I O N

INTRODUCTION

Foetus is totally dependent for its oxygen supply on the blood supplied through placenta. In any case if blood supply through placenta is hampered it leads to asphyxial injury. The incidence of perinatal asphyxia is about 1.0 to 1.5 percent in most centres and is usually related to gestational age and birth weight. It occurs in 9.0 percent of infants less than 36 weeks of gestation and 0.5 percent of infants of more than 36 weeks gestation, accounting for 20 percent of perinatal deaths or as high as 50 percent of deaths if still borns are included. The incidence is higher in term infants of diabetic or toxemic mothers. These factors correlate less well in preterm infants.

Ninety percent of asphyxial insult occurs in antepartum/intrapartum period as a result of placental insufficiency, resulting in an inability to provide O_2 and to remove CO_2 and H^+ from foetus.

During normal labour there is reduced blood flow to placenta, hence decreased O_2 delivery to the foetus. Because there is concomitant increase in O_2 consumption by both mother and foetus, foetal O_2 saturation falls. Maternal dehydration and maternal alkalosis from hyperventilation may further decrease maternal and foetal O_2 saturation. Some degree of cord compression also occurs

in many deliveries. Uterine contractions decrease placental blood flow. These events cause most babies to be born with little O_2 reserve.

In addition to the normal factors mentioned above any process which (1) impairs maternal oxygenation. (2) Decreased blood flow from mother to placenta or from placenta to foetus. (3) Impairs gas exchange across the placenta or at the foetal tissue. (4) Increases foetal O_2 requirement, will exacerbate perinatal asphyxia.

In the presence of hypoxic ischemic challenge to the foetus, reflexes are initiated causing shunting of blood to the brain, heart, and adrenals and away from lungs, gut, liver, kidneys, spleen, bone, skeletal muscle and skin (diving reflex).

In mild hypoxia there is decreased heart rate, slight increase in blood pressure to maintain cerebral perfusion, increased central venous pressure and little change in cardiac output. As asphyxia progresses; with severe hypoxia and acidosis, there is decreased heart rate, decreased cardiac output and initially increased then fall in blood pressure as oxidative phosphorylation falls and energy reserves become depleted. During asphyxia anaerobic metabolism produces lactic acid which because of poor perfusion remains in local tissues systemic acidosis may actually be mild until perfusion is restored and these local acid stores are mobilized.

Apgar score was developed to identify quickly the new born in need of resuscitation. It should be noted that Apgar score is partially dependent on the age maturity of the newborn. Immature infants are more likely to be hypotonic to have cynotic extremities and to have decreased responsiveness. Therefore, a score of 7 may be "Maximum" for a normal premature infant. Hypoxia in utero due to hypoperfusion, a fibrotic placenta, premature placental separation, or problems with the umbilical cord, may be responsible for low Apgar score. But it must be remembered that factors other than hypoxia in utero may affect the Apgar score as well as the infant's future prognosis. Such factors include prematurity, central nervous system (CNS) abnormalities cardiac and respiratory problems and maternal medications. Prior infection, abnormalities of development of the fetal CNS or insult to it, also may be a cause of perinatal asphyxia, perinatal problems and subsequent deficit.

In the past, the clinical course of the full term infant who experienced intrapartum asphyxia was thought primarily to reflect altered brain function, it is now known, however, that the infants can have a different clinical course due to variable involvement of various organ systems. The variation in clinical signs is due in part to the ability of the foetus to redistribute blood flow to protect vital organs. It has been documented that the infants with low Apgar score, have had problems with their

pulmonary, cardiovascular, central nervous, gastrointestinal and renal systems. The effects of asphyxia on the liver and the hepatic functions of the neonate is a relatively unexplored avenue. This study is being carried out to assess the effect of birth anoxia on hepatic functions of a neonate as also the ultimate outcome of these cases.

The liver plays a central role in the synthesis, degeneration and regulatory metabolism. Due to asphyxia the liver may be so damaged ("shock Liver") that it may not provide its basic functions. Among parameters of liver cell dysfunction the serum activity of glutamic pyruvic transaminase (SGPT) and glutamic oxaloacetic transaminase (SGOT) is most specific in adults and infants (Kove et al, 1957). The release of these enzymes from damaged tissue into the blood stream is the principal factor responsible for increased serum transaminase activity in the presence of hepatic cellular injury, due to both cellular necrosis and reversible injuries of permeability of cellular or intracellular membranes (King et al, 1959).

Therefore, during perinatal hypoxia which causes a reversible increase in cellular membrane permeability, there is a release into the blood stream of cytoplasmic enzymes (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, lactic dehydrogenase). In the presence of liver cell necrosis both the cytoplasmic and mitochondrial enzymes are increased. Since SGOT is

also present in myocardium, kidney and RBC while SGPT is prim arily released from the liver, therefore, this enzyme is more specific for liver damage or injury. Alkaline phosphatase also rises in liver damage but it is less specific and less sensitive.

AIMS OF STUDY

1. To assess the liver functions in healthy newborns.
2. To assess the liver function in severe birth asphyxiated babies and compare with the controls.
3. To collect the information about severity of liver damage and their prognostic value.
4. The drugs that are detoxified by the liver must have their levels monitored closely because in a damaged liver , the drug metabolism may be altered.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Asphyxia is an insult to the foetus or newborn due to lack of oxygen (hypoxia) and or lack of perfusion (Ischemia) to various organs. The effect of hypoxia and ischemia may not be identical, but they can be difficult to separate clinically. Both factors probably contribute to asphyxial injury.

Asphyxia is a major contributory factor for still birth and perinatal deaths. Studies in human to assess the long term effect of asphyxia has been inconclusive, largely because of the difficulty of separating asphyxia from other adverse influences which may operate during labour and affect subsequent outcome.

Boyle (1970) established that the ability of animal to survive in an environment free of oxygen varies with age; younger and more immature the infants is, greater is the tolerance to total deprivation of oxygen. This increased resistance of the foetus to anoxia has been related to the adequacy of cardiac glycogen stores and possibly to additional source of energy to foetal tissue.

Adaptive mechanisms to withstand anoxia possessed by foetus may be overcome if the hypoxic insult is sufficiently severe. In foetal monkey it is known that neurological damage occurs after about 15 minutes of asphyxia, in humans the critical period can only be

surmised, as hypoxia at birth is so often continuation of intrauterine hypoxia of unknown duration.

The general consensus is that the redistribution and preferential routing of oxygenated blood to the upper body via foramen ovale are two mechanisms which place foetal heart and brain in a privileged position with respect to the arterial supply of oxygen(Born et al,1954). However, the quantitative question concerning the effectiveness and limitations of the mechanisms remain to be answered. The cardiac output redistribution which has been observed experimentally has not been compared with theoretic requirement of preventing a decrease in the supply of oxygen to heart and brain. Therefore, it is not clear whether the foetal circulation response to hypoxia is an effective defence process and at what level of oxygenation it may become adequate. The difference of oxygen content between the blood that perfuses the upper and lower body has not been measured in chronic animal preparations or related to oxygen availability.

Asphyxia at birth may result from or be associated with many conditions. The mechanism of intrauterine or birth asphyxia common to these may be appreciated from a consideration of placental transfer of oxygen from mother to foetus and this initiation and maintenance of respiration at birth. The human placenta is haemochoreal in type. Foetal capillaries come into direct relationship with a pool of maternal blood thereby creating a large interface

for exchange of oxygen and carbon dioxide.

The duration of foetal hypoxia is equally important. Studies in foetal monkey have demonstrated that total neuropathologic state (Ranck, 1959). However, partial hypoxia with metabolic acidosis must be present at least 2 hours before neuropathologic damage can be anticipated. A precise measure of the duration of foetal asphyxia in the human foetus is generally not available because of the periodic nature of foetal blood gas and acid base assessment and the intervention that abnormal blood gas and acid base measurements now require. However, the importance of duration of foetal asphyxia was implied in a study of 60 children with biochemical evidence of intrapartum foetal asphyxia at delivery. Children with deficits had an episode of asphyxia that was more severe and prolonged than the children with normal motor and cognitive development.

Bartels et al (1962) have shown schematic representation of foetal and maternal circulation within the uterus. They demonstrated that oxygen tension in foetal blood is very much lower than that of maternal or uterine venous blood. Formerly it was postulated that resistance to the diffusion of oxygen in the placenta was responsible for this large discrepancy. Later experimental work suggests, however, that there is little barrier to diffusion in the placenta and that the large fall in oxygen tension from 90-100 mm Hg in maternal arterial

blood to around 28 mm Hg in umbilical venous blood is explained by the level that the placenta itself consumes a great deal of oxygen. Uneven distribution of maternal and foetal placental blood flow to the site of gaseous exchange may also be important. The oxygen tension of umbilical venous blood led to the suggestion that the foetus lives under condition of oxygen deficiency comparable to a climber at altitudes around 18000 to 21000 feet. The apparent disadvantage of a low PO_2 is partially offset, however, by the high oxygen capacity of foetal blood and the Bohr Effect, thereby incompletely saturated hemoglobin binds less oxygen in an acid environment than in a basic one. The latter effect facilitates oxygen transfer from maternal to foetal blood and increases the delivery of oxygen to foetal tissues. The difference in oxygen affinity between maternal and foetal blood in later pregnancy is a relatively less important factor favouring oxygen transport from mother to foetus in utero. Although the precise oxygen requirements of foetus in utero are uncertain it seems that under normal circumstances foetal oxygen supply is far from precarious and the foetus can withstand stresses such as severe anaemia in erythroblastosis fetalis. Intrauterine hypoxia could result from a decrease in amount of oxygen reaching the site of gaseous exchange (low maternal arterial PO_2 or O_2 content, impaired uterine blood flow) limitation of the area available for gaseous exchange, imbalance between maternal and umbilical blood flow within the placenta,

reduced oxygen carrying capacity of foetal blood or decreased tissue utilization of oxygen. Several of these factors may act concurrently.

Whenever, arterial oxygen content decreases, the oxygen supply to every organ to the body tend to decrease also. Guyon et al, (1964) have emphasized the concept that the circulatory system can compensate for this effect of hypoxia by increasing cardiac output. An optimal compensation would require that the product of blood flow arterial oxygen content be kept virtually constant in each region of the body.

An increase in cardiac output concomitant with hypoxia has been described in adult animals and under certain experimental conditions. According to Guyton et al (1964) this increase in cardiac output has not been a consistent finding and its magnitude has been less than that required to hold constant O_2 supply to the entire system. There is general agreement that an increase in cardiac output does not play a significant role in the response of the ovine foetal hypoxia, probably because normal foetal cardiac output is already at a relatively high level. During hypoxia foetus attempts to preserve the arterial supply of oxygen to three regions of the body only namely heart, CNS, and adrenal. Of these adrenal requirement is small and can be neglected. However, as the arterial oxygen content decreases, the blood flow necessary to maintain the supply of oxygen to the heart and CNS

increases hyperbolically and become a large fraction of cardiac output.

During vaginal delivery there is diminished gaseous exchange across the placenta (Kubli and Berg, 1965). They observed acid base data during labour and at the time of delivery of vigorous healthy babies and asphyxiated depressed babies. Oxygen saturation was remarkably low in both normal and depressed infants. They further demonstrated that pH was the main indicator of severity of asphyxia and the low pH in severely depressed infants represented both respiratory and metabolic acidosis.

The percent of umbilical blood flow going through the ductus venosus increased with foetal distress at a time when the umbilical blood flow was decreasing . A sphincter at the junction of the umbilical vein with the ductus venosus has been described in sheep and humans. Although there is conflicting evidence about the role of such a sphincter in modifying blood flow through the ductus venosus (Peltonon and co-workers, 1965 and Lind et al, 1966). More recently, nerve fibres from the anterior and posterior vagal trunks have been described passing to a thickening of muscular wall in the region of the junction of umbilical vein with the ductus venosus and to the proximal portion of umbilical vein. No nerve fibres were traced to the wall of the ductus venosus itself. If the thickening is a sphincter like mechanism involving part of the proximal

end of the umbilical vein innervated by vagal and ciliac plaxes fibres as the evidence would seen to indicate, afferent and autonomic fibres of both sympathetic and parasympathetic function are probably also involved. According to Rudolf et al (1967), the blood flow to the gastrointestinal tract, kidney, spleen, liver (hepatic artery) in the primate in general, is slightly lower than those reported in sheep. They observed no significant change in the blood flow to these organs with foetal distress.

Successful respiratory adaptation in the transition period between foetus and newly born is crucial to extrauterine survival. As gestation progresses, movements of foetal thorax are increasingly difficult to elicit, presumably because of mechanisms which limit the effectiveness in stimulating respiration by sensory and chemical changes in utero. At birth, medullary respiratory neurons are flooded by new sensory impulses, exteroceptive (pain, touch, temperature), proprioceptive (muscle tension, joint) and afferent baroreceptive following clamping of umbilical cord. The activities of respiratory neurons result in rhythmic phrenic and intercostal discharge impulses and increases sympathetic activity. It has been proposed by Purves (1964) that increased sympathetic activity activates the peripheral chemoreceptors which inturn become involved in the regulation of respiration. Successful adaptation presupposes that the lungs can expand and become

functionally competant soon after birth in the absence of gross neurological or cardiovascular defects.

In the study of Herbert et al (1974), it was not possible to separate various factors which produce the vascular responses observed. The changes could be the result of direct local effects of hypoxemia and acidemia, increased autonomic nervous system activity and secretion of catecholamines and possibly the liberation of other vasoactive substances. Since maturation in the foetus of parasympathetic and alpha and beta sympathetic regulation is variable (Vapaavouri et al, 1973) it is possible that autonomic responses to hypoxemia may differ between species as well as with gestational development. There is also suggestive evidence that the adrenal gland secretes norepinephrine in the younger foetuses and a mixture of norepi. nephrine and epinephrine in later gestation.

Herbert et al (1974) showed that the foetal arterial blood pressure increased inresponse to maternal hypoxia, and this was particularly significant in the foetuses which developed hypoxemia and acidemia. Since combined ventricular output either did not change or fell, the hypertensive response was related to increased peripheral resistance. The vascular systems that participated in producing this increased resistance were those of the carcose, gut kidney, spleen and to some extent the lung. Although the umbilical placental correlation has been

thought to be relatively unresponsive to the degree of hypoxemia produced in foetuses, the fact that the systemic arterial blood pressure increased consistently and umbilical flow did not change significantly suggests that there was some degree of umbilical placental vascular constriction. Although foetal central venous pressure did not increase during the hypoxemic period, the workers of the study did not know whether umbilical venous pressure increased or not as it was not measured. It is possible that the apparent increase in umbilical placental vascular resistance could be related to an increase in umbilical venous pressure, possibly associated with increased impidance in the ductus venosus or hepatic portal circulation.

Foetal adaptation to hypoxemia was accomplished predominantly by redistribution of combined ventricular output in order to maintain blood flow to vital organs such as placenta, heart, brain and adrenal glands, while flow to other organs, the skin, the musculoskeletal system, decreased. Further more the magnitude of the change of flow in the organs could have been influenced by the experimental manipulations such as anaesthesia and acute surgical stress. Thus it is apparent that the foetal circulatory responses to hypoxemia may be influenced not only by gestational maturation but also by mechanism of production of hypoxemia.

The same findings were observed by Cohn et al

(1974) who observed that the foetal response to hypoxemia is the key to the effect of the degree and duration of foetal asphyxia. Foetal hypoxemia results in an increase in arterial pressure due to increased vascular resistance. This is associated with redistribution of cardiac output characterised by reduced blood flow to the pulmonary, gastrointestinal circulation and the body with increased blood to the heart, brain and adrenal glands. However, the magnitude of the circulatory adjustment which is required by a certain level of oxygenation can be understood more clearly by focussing attention on arterial oxygen content.

The degree of asphyxia is relevant in regard to outcome. Neuropathologic findings in the foetal monkey in response to total anoxia were different from those after partial asphyxia (Myers, 1975). The human foetus in the clinical setting may develop a significant degree of metabolic acidosis because of severe hypoxemia acting over a short period or milder degree of asphyxia acting over a longer period.

According to Peeters and associates (1979) during moderate hypoxia, the blood flow to parts of the foetus like kidneys may also increase to some extent. Nevertheless the experimental points agree with the concept that the circulatory response of the foetus to hypoxia is centered on the requirement of maintaining a constant flow of oxygen to the heart and CNS without any

marked increase of cardiac output.

Each cardiac output is subdivided into four main sections. 1. Placental flow, 2. Lung flow, 3. Flow to CNS, and 4. Flow to the remainder of the body. These sections corrsspond closely to the four groups of tissues that respond differently to oxygen variability (Peeters et al, 1979). They found that the most consistent change of cardiac output distribution was the reciprocal relationship between oxygen content in ascending aorta and percentage of cardiac output directed to heart and CNS. They found lack of sufficient association between cardiac output and oxygen and suggested that there should be a reciprocal relationship between blood flow to other parts of body. In agreement with this suggestion there will be a significant negative correlation between blood flow to the CNS and heart and blood flow to the rest of foetal body.

Umbilical placental blood flow is maintained when foetal hypoxia is a result of maternal hypoxemia or reduced maternal uteroplacental blood flow (Parer, 1980).

Foetal hypoxia due to cord occlusion is associated with decreased umbilical placental blood flow. However, blood flow to the central circulation is maintained through the ductus venosus with a marked reduction of blood flow through the liver (Isskowitz and co-workers, 1982).

The autonomic nervous system is principally responsible for the increased vascular resistance and redistribution of cardiac output. There is evidence to indicate that the response is initiated through arterial chemoreceptors and may be influenced by circulating endogenous opiates (La Gamma, 1982). Other factors may include increased angiotensin activity and release of vasopressive (Start et al, 1982).

The target organs of perinatal asphyxia are the brain, heart, lung, kidney, liver, bowel and bone marrow. The most frequent abnormalities involved the kidney (50 percent), followed by the CNS (28 percent), cardiovascular (25 percent) and pulmonary (23 percent) systems. Often asphyxiated infants will succumb to dysfunctions of organs other than CNS (persistent foetal circulation) while showing minimal evidence of hypoxic ischemic brain injury. In such instances brain was spared at the expense of cardiac output to the affected organ (Brann, 1986). The degree of asphyxia required to cause permanent neurological impairment is just below that which is lethal from multi-system failure (Freeman et al, 1988).

The liver may be so damaged (shock liver) that it may not provide its basic functions. In such cases, liver function tests (transaminases SGOT, SGPT) clotting factors (PT, PTT, fibrinogen), albumin and bilirubin should be monitored. If total liver failure occurs it is a usually a bad prognostic sign. Otherwise, the cardiac,renal,

gastrointestinal, pulmonary, hepatic and haematological problems usually resolve if the infant survives.

In recent study of asphyxiated newborns by Perlman (1989), 24 percent had no evidence of organ injury, 23 per cent had an abnormality confined to one organ, 34 percent involved two organs and 9 percent affected three organs.

Saili et al (1989) studied 46 full term neonates. Out of them, 31 neonates had suffered from severe birth asphyxia. They found in their study that liver was damaged as a result of perinatal asphyxia leading to increase in transaminase levels in serum. These levels were significantly higher in neonates who died due to asphyxia as compared to the one who survived.

LIVER FUNCTIONS AND SERUM ENZYMES

Clinician first became interested in serum enzymes more than 40 years ago when the role of alkaline phosphatase in the diagnosis of hepatobiliary disease was reported (Roberts, 1933).

Enzymes are found inside cell, where they catalyse numerous biochemical reactions. Normally they enter the blood in small amounts, as a result of natural cell turn over and perhaps also by diffusion through undamaged cell membranes (Baron, 1964).

Elevated serum activity are invariably found in clinical states in association with extensive tissue necrosis, Raised serum enzyme activity however, do not

always indicate widespread cell death, since marked elevation are commonly present in reversibly inflammatory states (Wilkinson, 1977).

SERUM GLUTAMIC OXALOTRANSAMINASE (SGOT) AND SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT)

General Consideration

The determination of the activity of SGOT offers a simple and rapid laboratory test for establishing the presence of acute hepatocellular damage or dysfunction. This enzyme had a wide spread distribution in animal and human tissues and is also present in normal serum and plasma (Cohn et al, 1941, Awapera et al, 1952).

The widespread use of enzyme tests followed the observation of Karmen Wrobelwski et al (1953), that transaminase activity waspresent in serum of normal individual and that raised activity may reflect injury, not only to liver but also to other organs.

SGOT exist in two intracellular forms, one in the cytosol and the other in mitochondria. Only one form of SGPT is known and liver is by far its richest source. The two isoenzymes of SGOT have been separated electrophoretically. The ratio of cytosol to mitochondrial enzyme is said to be correlated well with the severity of liver damage (Franchis et al, 1972). Higher activity of SGOT is recorded in early life than there after and Kattwinkel et al (1973) found the SGOT, unlike SGPT, to be age related between the years of 4 and 30.

According to Felix Wroblewski (1959) the mean SGOT activity of serum of normal persons determined by the calorimetric technics is 16±8.0 units,,with a normal range of 4 to 40 units. While that of SGPT is 22±12 unit, with a normal range of 1 to 45 units. In all instances the activity of SGOT in any one tissue is greater than that of SGPT. In the case of SGOT the greatest activity is observed in extracts of skeletal, diaphragm and heart muscle and liver tissue.

The activity due to transaminase in serum of adults, both in physiologic and pathologic states, had been well documented, little information relative to the behaviour of the enzyme in the newborn infant is available (Abelson, 1956).

Simon Kove et al (1957) determined the normal activity of SGOT in the serum in the early neonatal period and compared it with that of adult values. They measured activity of SGOT in serum of cord blood of nine newborns, forty six newborns between the age of 1-5 days and eight newborns between the age of 6-11 days. Infants were chosen randomly. Infants with physiological jaundice were included. They obserbed a range of 13-105 units of SGOT in the newborn period (with the exception of one infant in whom the level was 160 units). This is considerably wider range than that of 5-45 units found in normal adults and a value more than 45 units in adults is considered abnormal

and raises a strong suspicion of the presence of some pathologic state. According to them the activity of SGOT was not related to the age of infant within neonatal period studied and varied widely in different infants for each day of age, without any distinctive pattern. Variations of the activity of SGOT in specimens of cord blood studied ranged below the 59 units, which was lower than for any other day of neonatal period adequately investigated. No relation was found between the concentration of bilirubin and the activity of SGOT in the serum of normal neonates.

A rise in serum transaminase activity occurs in most hepatocellular disorders. These tests are most sensitive indicators for liver cell damage. By contrast rise in transaminase is much less constant in chronic hepatocellular disorders. Less than one third of adult patients with well compensated cirrhosis for example, have increased SGOT activity (Foulk, 1964). The cells of several organs contribute the SGOT activity, because the liver is the prime source of SGPT, measurement of this enzyme is more specific for liver damage. Hence during viral hepatitis there is a proportionately greater rise in SGPT activity. This increases by 20 to 100 folds in more than ninety per cent of patients, while in eighty percent SGPT rises by only about 10 to 20 folds. The ratio of SGPT : SGOT therefore is about 1.2, whicl falls during infective hepatitis to about 0.2 (De Ritis et al, 1972). Finally

SGOT and SGPT provides no clue to prognosis, in either acute or chronic liver disease, since they do not measures the extent of hepatocellular damage. In acute hepatocellular disease, the electrophoretic separation of the SGOT isoenzyme affords an indication of the severity of liver damage. In massive necrosis for example, large amounts of mitochondrial enzyme are released into circulation. Conversely because this isoenzyme has a short half life, its disappearance from the serum heralds recovery(Wilkinson, 1970).

SGOT AND SGPT IN BIRTH ASPHYXIA

In the presence of liver cell necrosis both the cytoplasmic and mitochondrial enzymes are increased (Isselbacher et al, 1983).

Fits Simons et al (1984) in their study reported an increase in SGOT in asphyxiated neonates while no significant increase was observed in the values of alkaline phosphatase.

According to Vincenzo Zanardo et al (1985) the serum activity of SGOT and SGPT is one of the most specific parameter of liver cell injury in adults and infants. The release of these enzyme from damaged tissues into the blood stream is the principal factor responsible for increased serum transaminase activity in the presence of hepatic cellular injury due to both cellular necrosis and reversible injuries of permeability of cellular or intracellular membranes. They showed that during perinatal hypoxia which

causes a reversible increase in cellular membrane permeability, there is a release into the blood stream of cytoplasmic enzymes (SGOT and SGPT). In the presence of liver cell necrosis both the cytoplasmic and mitochondrial enzymes are increased. In their study they examined the behaviour of SGOT and SGPT activity in full term and preterm newborns. The mean value of SGOT activity was 50 units/l and seemed to decrease over the first 30 days of life.

During first 72 hours of life in full term asphyxiated newborns there was a significant increase in mean SGOT activity as compared to full term healthy newborns (Mean$\pm$S.D. - 100$\pm$68.9 and 52$\pm$12.9 respectively; $p < 0.01$). Moreover, in full term asphyxiated newborns the mean values of SGOT activity were significantly higher than in preterm asphyxiated newborns (100$\pm$68.9, 59.2$\pm$29.1 respectively, $p < 0.005$) whereas there were no statistical difference between healthy full term and preterm newborns (52$\pm$12.9, 50.1$\pm$13.4 respectively), nor between healthy and asphyxiated preterm newborns (50.1$\pm$13.4, 59.2$\pm$29.1 respectively). Between 5th and 10th day of life in full term asphyxiated newborns the mean value of enzymatic SGOT activity decreased.

In healthy full term and preterm newborn, the mean value of SGPT serum activity was 25 U/l and was lower than in asphyxiated full term newborns, which was 54.5 U/l During first 72 hours of life, in full term asphyxiated newborns there was a significant increase in the mean SGPT activity in comparison with healthy full term newborns

(mean ±SD = 54.5±54.4, 18±6.6 respectively, p <0.025). Moreover, in full term asphyxiated newborns the mean values of SGPT activity were significantly higher than in preterm asphyxiated newborn (54.5 ±54.4, 11.8±8.2 respectively, p <0.0001), as well as in full term healthy newborn when compared with preterm healthy newborns (18±6.6, 11.1±5.8 respectively, p <0.005). There was no significant difference between healthy and asphyxiated preterm newborns (11.1±5.8, 11.8±8.2 respectively).

Between 5th and the 10th day of life in full term asphyxiated newborns the mean values of SGPT remained significantly higher than in full term newborn, as well as in full term asphyxiated newborn in comparison with pre term asphyxiated newborns.

Saili et al (1989) studied liver function tests in 46 full term neonates. Out of this 31 infants had suffered severe birth asphyxia, while 15 normal babies formed the control group. They showed that liver function tests (SGOT and SGPT) were deranged in asphyxiated babies. According to them deranged levels of SGOT and SGPT were noted in 64.52% of asphyxiated babies. There was 60% mortality in asphyxiated babies with deranged liver functions. The serum levels of transaminases in non survivors were significantly higher than those of survivors. The SGOT level among controls was in the range of 54.83± 48.86 IU/l while in asphyxiated babies 97.84±119.42 IU/l. The SGPT level among controls was in the range of 22.4± 32.96 IU/l while in asphyxiated babies in the range of

44.09±61.94 IU/l.

Therefore knowledge of behaviour of SGOT and SGPT activity may have important implication in the diagnosis and early treatment of perinatal asphyxia.

ALKALINE PHOSPHATASE

The name alkaline phosphatase applied to a group of enzymes which, acting optimally at alkaline pH, catalyse the hydrolysis of several organic phosphate ester with liberation of inorganic phosphate. The total serum alkaline phosphatase activity is the sum of various distinct tissue components which may be separated by electrophoresis. In the fasting individual both hepatic and intestinal isoenzymes are present, the latter enzyme is enhanced following ingestion of a lipid meal and is greater in individuals who are secretors of blood group B and O (Langman et al, 1966). A third fraction, of osseous origin, is present in the serum of children particularly. The relative proportion of this fraction in total alkaline phosphatase activity at different ages is important. Less significant amount of alkaline phosphatase activity is also present in kidney, leucocytes and neoplastic tissues.

Unlike the total alkaline phosphatase activity in adults, that recorded during childhood and adolescence is principally of osteoblastic activity and is, therefore, closely related to age. During periods of rapid skeletal growth relatively high activity is usual. Thus from birth

to 12 months of age. Clarke and Beck (1950) reported values upto 50 percent higher than the mean in the middle years of childhood.

Alkaline phosphatase activity closely mirrored the growth velocity. Round (1973) showed a significant alkaline phosphatase rise in boys of 10 to 14 years ($p < 0.0005$) which paralleled the adolescent growth spurt, a less obvious rise occurred in girls between 8 and 12 years ($0.005 > p > 0.0001$).

Within liver itself more than one form of alkaline phosphatase exists. In the rat for example, two iso-enzymes have been identified , one in the cytosol, the other bound to nuclear or microsomal membranes (Simons and Sutherland, 1973).

Total alkaline phosphatase is a nonspecific determination whose major contribution is derived from bone and liver. Therefore, its value in the differential diagnosis of infantile liver diseases is very limited (Mowat et al, 1976). Biliary obstruction, particularly if protracted, tend to be associated with high values, while in less prolonged biliary obstruction or in hepato-cellular jaundice, only mild raised alkaline phosphatase activities are recorded (Backer and Stauffer, 1962).

ALKALINE PHOSPHATASE IN BIRTH ANOXIA

In clinical practice, total alkaline phosphatase lacks the sensitivity of a good screening test for liver

disease. In a group of children known to have hepato-biliary dysfunction, Belfield and Goldberg (1971) found that 27 percent had normal activity.

Clinically the increase in alkaline phosphatase activity is clearly not related to hepatocellular damage, since even total hepatic necrosis accounts for only about two folds rise (Ritt et al, 1969).

According to Saili et al (1989) there is rise in alkaline phosphatase in severely asphyxiated newborns. According to them serum alkaline phosphatase in asphyxiated group was 17.64±12.30 KAU/dl as compared to 14.36±9.06 KAU/dl in controls.

SERUM BILIRUBIN

Bilirubin in the blood is produced from the ferroporphyrin, haem, after removal of its iron component. Eighty percent is derived from haemoglobin breakdown by Kupffer cells in the liver and other macrophages in the spleen and liver.

Within the hepatic cell, bilirubin is conjugated to bilirubin glucuronide, Schmid (1956) and Billing et al (1957).

$$2 \text{ UDP glucose} + 2 \text{ NAD} \xrightarrow[\text{dehydrogenase}]{\text{UDP glucose}} 2 \text{ UDP glucoronic acid} + 2 \text{ NADH} + 2 \text{ H.}$$

$$2 \text{ UDP Glucoronic acid} + \text{Bilirubin} \xrightarrow[\text{transferase}]{\text{UDP glucuronyl}} \text{Bilirubin diglucoronide} + 2 \text{ UDP.}$$

The serum bilirubin tends to be low in the fetal circulation and rises sharply in the newborn infant shortly after birth (Davidson et al, 1941). Since both UDP glucose dehydrogenase and UDP glucuronyl transferase are very low in fetal liver (Brown and Zuelzer, 1958) conjugation of bilirubin to its glucuronide cannot occur in the fetus at a normal rate. Therefore, the bilirubin must be removed from the fetal circulation by transfer across the placenta to the maternal circulation and eventually must be conjugated by the maternal liver and excreted in her bile.

In 1959, Schmid et al carried out preliminary investigations on the placental transfer of bilirubin in pregnant guinea pigs during last weeks of gestation. On the maternal side, a uterine vein was connulated which permitted continuous sampling of venous blood from an individual placenta. On the fetal side, an umbilical artery was cannulated while the umbilical vein was served and the cord clamped proximal to the fetus.

As soon as the cord is cut, the fetus loses the placental mechanism for the removal of bilirubin through the maternal liver and bile. As a result there is a moderate accumulation of unconjugated bilirubin in the plasma.

Brown and Zuelzer (1958) reported a marked decrease of UDP glucose dehydrogenase and UDP glucuronyl transferase activity in the fetal and newborn guinea pig.

In older children and adults the concentration of serum bilirubin is 0.2 to 0.8 mg/dl, although in normal adults, values as high as 1.5 mg/dl may be found (Zieve and Still, 1955). In the mature newborns, however, levels upto 6 mg/dl are recorded on the 2nd to 4th days of life, while the preterm baby 5 to 7 days after birth, often shows values of 12-14 mg/dl.

The total bilirubin concentration is rarely of value in differentiating the cause of jaundice. Although much over lap occurs between values found in various types of jaundice. Outside the newborn period it is generally agreed that simple haemolysis rarely results in levels greater than 5 mg/dl. Similarly the bilirubin level does not correlate with the severity of liver disease.

An elevated direct reacting bilirubin is a more sensitive indicator of mild or early liver injury than an increase in the total serum bilirubin. In neonatal period, however, this finding is less specific since a rise in conjugated bilirubin is found in serious, pyogenic non hepatic infection (Hamilton et al, 1963).

Serum concentration of bilirubin represents a balance between its production and metabolism. Hyper-bilirubinemia therefore, may be the result of over production of bile pigment or impaired hepatic uptake, conjugation or excretion (Arios, 1974, Maisels, 1975).

A raised bilirubin level lacks liver specificity, since an increase may occurs in haemolysis. In the newborns

the physiological rise of bilirubin, may be exaggerated by several abnormalities not caused by primary hepatic dysfunction; these include hyperthyroidism, a large haematoma, or a high bowel obstruction, in each of which the bilirubin is unconjugated (Johnson, 1975).

A study carried out by Simon Kove et al (1957) illustrated that hyperbilirubinemia, which is normally present in the neonatal period is not associated with increased activity of SGOT. He demonstrated a case where the activity of SGOT was only 28 units, despite marked clinical icterus and the concentration of bilirubin in the serum was 33 mg/dl. In contrast to this infant, others with low concentration of bilirubin often displayed increased activity of SGOT in the serum.

SERUM BILIRUBIN IN BIRTH ASPHYXIA

Saili et al (1989); in their study compared serum bilirubin in severe asphyxiated newborns with normal newborns. According to them mean serum bilirubin level in control and asphyxiated newborns was 4.50 ± 6.12 and 4.78 ± 6.62 mg/dl respectively. But due to multifactorial etiology of serum bilirubin, they were not able to conclude that this rise in serum bilirubin was due to asphyxiated damage of liver.

APGAR SCORE

The Apgar score devised in 1952 by Dr. Virginia Apgar, is a quick method of assessing the state of newborn

(Curr Res Anaesthe Analg, 1953). Although Apgar score continues to provide a convenient short hand for reporting the state of the baby and the effectiveness of resuscitation. The purpose of this statement is to place the APGAR score in its proper prospective as a tool for assessing asphyxia and for prognostication. The Apgar score was developed to identify quickly the newborn in need of resuscitation (Apgar, 1953).

The Apgar score is comprised of five components heart rate, respiratory effort, tone, irritability and colour. Each of which can be given a score of 0, 1 and 2.

Component	Score		
	0	1	2
1. Heart rate	Absent	Slow (<100 beats/min.	>100 beats/min.
2. Respiration	Absent	Weak cry, hypo-ventilation	Good strong cry
3. Muscle tone	Limp	Some flexion	Active motion
4. Reflex	No response	Grimace	Cough or sneezing
5. Colour	Blue or pale	Body pink extremities blue	Completely pink

It is important to recognise that elements of the score such as tone, colour and reflex irritability are partially dependent on the physiologic maturity of the infant. The normal premature infant may thus receive a low score purely because of immaturity with no evidence of anoxic insult or cerebral depression.

One minute Apgar score : A low one minute Apgar score indicates an infant who may need resuscitation. Although a score of less than 6 is listed in the international classification of diseases, revision 9, codes as asphyxia, a low score at one minute neither indicates that substantial hypoxia or ischemia has occurred not has much prognostic significance.

In the collaborative perinatal project 4.8 per cent of surviving infants has a one minute Apgar score of 3 or less (Nelson et al, 1979). The one minute Apgar score should not itself be used as indication of prior asphyxia or as a predictor of future deficit.

Five minute Apgar score : The Apgar score at five minutes indicates the infant who needs continued resuscitative efforts. The score is affected by all the conditions noted to affect the one minute Apgar score.

Ten Minute Apgar score : An Apgar score that continues to be 3 or less at 10 minutes indicates that the infant has remained hypoxic or hypoperfused despite resuscitative efforts. Only a small fraction of one per cent of all full term infants in the collaborative perinatal project had such a score. Of these 34 percent died during the first year. However, if they survived most of these infants did well.

Fifteen and Twenty minute Apgar score : A score of 3 or less at fifteen or twenty minutes after delivery despite resuscitative efforts indicates that the full term

infant has suffered a severe anticedent injury with the possibility of additional postnatal effect. Often but not always this may be a result of intrauterine hypoxia. The mortality rate of these infants is 53 percent and 59 per cent respectively. Failure of low scores to increase at 5, 10 or 20 minutes indicates an on going insult that could affect, or further affect outcome. Continued low scores at 10, 15 and 20 minutes are associated with increasing mortality and long term morbidity. However, only a small fraction of 1 percent of infants had a score of less than 3 at 20 minutes and survived. Rapid improvements of scores by five to ten minutes indicate that the prior insult was unlikely to have been sufficiently severe to result in permanent deficit (Nelson, Elbenberg, 1981).

In an infant with a low Apgar score, umbilical cord acidemia in the absence of maternal acidemia, large base deficit, and presence of nucleated RBC, in the peripheral blood provide supporting evidence of asphyxia. Liver, renal and cardiac dysfunction may also provide evidence of asphyxia.

It should be noted that Apgar score partially depends upon the maturity of the newborn (Catlin et al, 1986). Immature infants are more likely to be hypotonic to have cyanotic extremities, and to have decreased responsiveness. Therefore a score of 7 may be "Maximum" for a normal premature infant.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College, Hospital, Jhansi from September, 1993 to August, 1994. The cases included in the study were divided into two groups i.e. study and control groups.

SELECTION OF CASES

Control group (A)

Fifteen normal full term neonates appropriate for gestational age were included in this group. These neonates did not have perinatal asphyxia or evidence of any liver disease.

Study group (B)

Full term neonates with severe birth anoxia were selected for study group. Babies having significant problems viz. significant congenital anomalies, septicemia, history of leaking more than 12 hours or absent membrane, heart disease, renal failure, hepatosplenomegaly, preterm neonates, twins and low birth weight neonates were excluded both from study and control groups.

DIAGNOSIS OF SEVERE BIRTH ANOXIA

Diagnosis of severe birth anoxia was made when Apgar score was ≤ 3 and ≤ 5 at 1 and 5 minutes respectively.

After selection of cases detailed history, clinical examination and investigations were recorded in

predesigned proforma as given below :

PREVIOUS OBSTETRIC HISTORY

It was enquired particularly history of previous abortion, jaundiced baby, birth asphyxia, obstructed labour, heart disease, congenital anomaly, blood group incompatibility.

ANTENATAL HISTORY

Any history of pre-eclampsia, eclampsia, leaking, diabetes mellitus, antepartum haemorrhage, convulsions, exposure to radiation, infection, fever with rashes, drug intake, date of last menstrual period or any other ailment during antenatal period were noted.

NATAL HISTORY

History of presentation, mode of delivery, any medication given during delivery and examination suggestive of foetal distress was noted and monitored.

POSTNATAL HISTORY

History of any medication given after delivery and Apgar score at 1 and 5 minute were noted.

EXAMINATION OF BABY

Each baby was subjected to thorough examination especially for any congenital anomalies, convulsions, septicemia, asphyxia, cardiovascular system, central nervous system and any other abnormalities.

Weight of each newborn infant was recorded with electronic weighing scale.

The assessment of gestational age was done by recording the last date of menstrual period and confirmed by physical and neurological developmental score (Modified scoring system for assessment of gestational age in newborn by Meharban Singh et al, 1975).

INVESTIGATIONS

Blood samples were drawn after 48-72 hours of birth and following investigations were done :

Serum bilirubin	Alkaline phosphatase
S.G.O.T.	S.G.P.T.

Other investigations whenever needed.

Collection of blood samples

Five millilitre blood was drawn from peripheral vein of neonate between 48-72 hours after delivery taking all aseptic precautions and taking informed consent from their attendants. Blood was collected in plain vial for liver function tests. Sample was centrifuged on the same day, serum was separated and preserved in deep freezer for liver function tests.

ESTIMATION OF LIVER FUNCTION

The liver function tests - serum bilirubin, SGOT, SGPT and Alkaline phosphatase were estimated by diagnostic chemical kits (SPAN).

1. SERUM BILIRUBIN

Principle

The basic principle is that bilirubin is diazotized only in presence of its dissolving solvent (Methanol). Thus the red purple coloured azobilirubin produced in presence of methanol originates from both direct and indirect fractions and thus represents total bilirubin concentration.

The intensity of red-purple colour so developed is measured colorimetrically. It is proportional to the concentration of the appropriate fraction of bilirubin. This reaction can be represented as :-

Bilirubin + Diazotized sulfanilic acid $\xrightarrow{H+}$ Azobilirubin $\downarrow$ H+ Red purple colour (λ max : 540 nm)

Reagents

Reagent 1 : Diazo-A
Reagent 2 : Diazo-B
Reagent 3 : Diazo blank
Reagent 4 : Methanol
Reagent 5 : Artificial standard (= 10mg% bilirubin).

Preparation of working solution

Diazo reagent was prepared by mixing 3.3 ml of reagent 1 with 0.1 ml of reagent 2.

Procedure

Clean, dried test tubes labelled total(T_1) and Blank (T_2) were arranged. Amount of reagents pippetted

into each tube was as follows :

Reagents	T_1	T_2
Serum (ml)	0.2	0.2
Distilled water (ml)	1.8	1.8
Reagent 3 : Diazo blank (ml)	-	0.5
Diazo reagent (ml)	0.5	-
Reagent 4 : Methanol (ml)	2.5	2.5

Contents of the test tubes were thoroughly mixed and the tubes T_1 and T_2 were kept in dark at room temperature for 30 minutes and optical density was read against distilled water on a colorimeter using yellow green filter.

Standard

Optical density of reagent 5 (Artificial standard = 10% bilirubin) was noted against distilled water.

Calculation

$$\text{Total serum bilirubin concentration (mg/dl)} = \frac{\text{Optical density of } T_1 - \text{Optical density of } T_2}{\text{Optical density of standard.}} \times 10$$

2. ALKALINE PHOSPHATASE

Principle

Alkaline phosphatase present in serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with aminoantipyrine in presence of oxidising agent potassium

ferricyanide and forms an orange-red coloured complex, which can be measured colorimetrically. The colour intensity is proportional to the enzyme activity.

The reaction can be represented as :

Phenyl phosphate $\xrightarrow[\text{pH } 10.0]{\text{Alk. Phosphatase}}$ Orange-red coloured complex.

Reagents

Reagent 1 : Buffered substrate pH 10.0
Reagent 2 : Sodium hydroxide, 0.5 N
Reagent 3 : Sodium bicarbonate, 0.5 N
Reagent 4 : 4-Aminoantipyrine, 6%,
Reagent 5 : Potassium ferricyanide, 2.4%
Reagent 6 : Stock phenyl standard 10 mg%.

Preparation of working solution

Solution I : In vial of reagent 1, 3 ml of distilled water was added and mixed well.

Solution II : Vial of reagent 4 was dissolved in 100 ml distilled water.

Solution III : Vial of reagent 5 was dissolved in 100 ml of distilled water.

Working standard: Vial of reagent 6 was diluted with distilled water in the strength of 1:10.

Procedure

Clean, dried test tubes were taken and labelled as Blank (B), Standard (S), Control (C) and Test (T). Test tubes were arranged and amount of reagents pippetted into each tube was as follows :

Reagents	B	S	C	T
Solution I (ml)	-	-	1.0	1.0
Distilled water (ml)	2.1	2.1	1.0	1.0

All the test tubes were well mixed and incubated at 37°C for 3 minutes.

Working standard (mL)	-	1.0	-	-
Serum (ml)	-	-	-	0.1

Test tubes were well mixed again and incubated at 37°C for 15 minutes.

Reagent 2 (ml)	0.8	0.8	0.8	0.8
Serum (ml)	-	-	0.1	-
Reagent 3 (ml)	1.2	1.2	1.2	1.2
Solution II (ml)	1.0	1.0	1.0	1.0
Solution III (ml)	1.0	1.0	1.0	1.0

All the test tubes were well mixed after addition of each reagent and optical density of Blank (B), Standard (S), Control (C) and Test (T) was measured against distilled water using a green filter.

Calculation

$$\text{Serum Alk. phosphatase activity in KA units.} = \frac{\text{Optical density of test - optical density of control}}{\text{Optical density of standard - optical density of blank.}}$$

3. S.G.O.T.

Principle

SGOT catalyses the following reaction :

$$\text{Alpha ketoglutarate} + \text{L-aspartate} \rightleftharpoons \text{L-glutamate} + \text{Oxaloacetate}$$

Oxaloacetate so formed is coupled with 2, 4-dinitrophenyl hydrazine (2, 4 DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline

medium and this is measured colorimetrically.

Reagents

Reagent 1 : Buffered aspartate - alpha - KG substrate pH 7.4
Reagent 2 : DNPH colour reagent.
Reagent 3 : Sodium hydroxide, 4 N
Reagent 4 : Working pyruvate standard, 2 mM

Preparation of working solution

Solution I : 1 ml of reagent 3 was diluted in 10 ml of distilled water.

Procedure

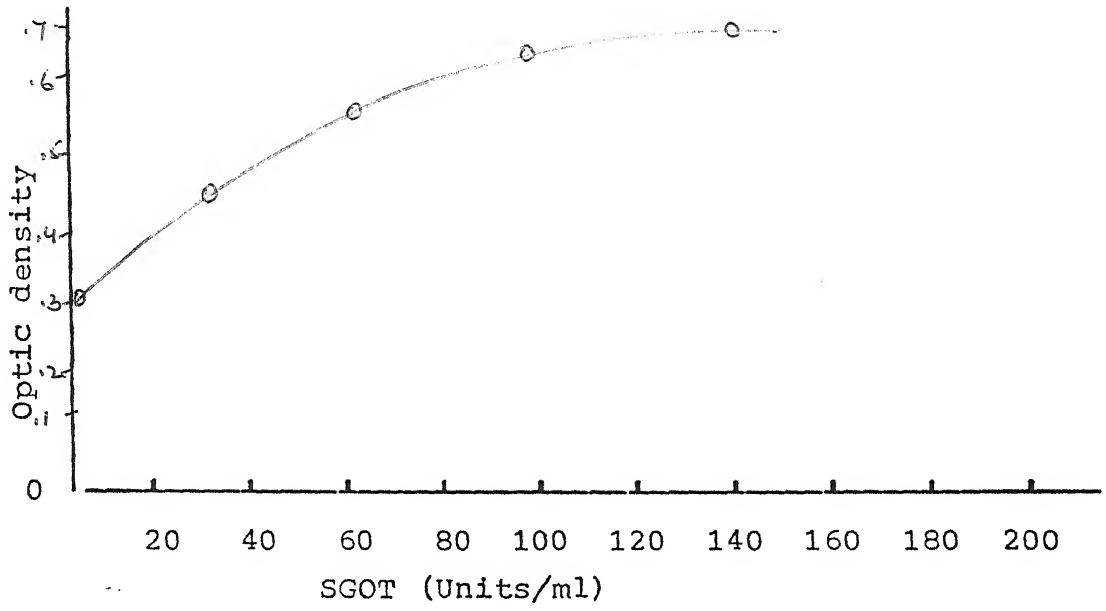
As the reaction proceeds with time, more amount of products are formed and since the end products inhibit the enzymes, there is more of inhibition. Because of this problem, it is necessary to standardize the calorimetric method against a standard kinetic method. In this kit, this standardization is done against the standard Karmen Unit Assay (Kinetic).

Clean, dry test tubes were taken and labelled as 1, 2, 3, 4, 5 and T. Test tubes were arranged and amount of reagents pippetted in each tube as follows :

Reagents	Test tube number				
	1	2	3	4	5
Enzyme activity(Unit/ml)	00	24	61	114	190
Reagent 1 (ml)	0.5	0.45	0.4	0.35	0.3
Reagent 4 (ml)	-	0.45	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
Reagent 2 (ml)	0.5	0.5	0.5	0.5	0.5
All the test tubes were well mixed and allowed to stand for 20 minutes at room temperature.					
Solution I (ml)	5.0	5.0	5.0	5.0	5.0

Test tubes were well mixed by inversion. These were allowed to stand at room temperature for 10 minutes and optic density of all five test tubes was measured against distilled water on colorimeter with a green filter.

Standard graph was plotted taking enzyme activity on X axis and optic density on Y-axis.



Test

Reagent 1 : 0.5 ml (Incubated at 37°C for 5 minutes)

Serum : 0.1 ml (Mixed well and incubate at 37°C for 60 minutes).

Reagent 2 : 0.05 ml (Mixed well and allowed to stand at room temperature for 20 minutes).

Solution I : 5.0 ml

Test tube was mixed well and allowed to stand at room temperature for 10 minutes and the optic density was read against distilled water on colorimeter using a green filter.

Calculation

The optic density of test was marked on the Y axis of the standard curve and extrapolate it to the corresponding enzyme activity on X - axis.

4. S.G.P.T.

SGPT catalyses the following reaction :

Alpha-ketoglytarate + L-Alanine = L-glutamate + pyruvate

Pyruvate so formed is coupled with 2,4-Dinitrophenyl hydrazine to give the corresponding hydrazone, which gives brown colour in alkaline medium and this can be measured colorimetrically.

Reagents

Reagent 1 : Buffered alkaline-Alpha-KG substrate pH 7.4

Reagent 2 : DNPH colour reagent

Reagent 3 : Sodium hydroxide, 4 N

Reagent 4 : Working pyruvate standard, 2 mM.

Preparation of working solution

Solution I : 1 ml of reagent 3 was diluted in 10 ml of distilled water.

Procedure

As the reaction proceeds with time, more amount of products are formed and since the end products inhibit the enzyme there is more inhibition. Because of this problem, it is necessary to standardize the colorimetric method against a standard kinetic method. In this, kit

standardization is done against the standard Karmen Unit Assay (Kinetic) and this is extrapolated to different amounts of pyruvate.

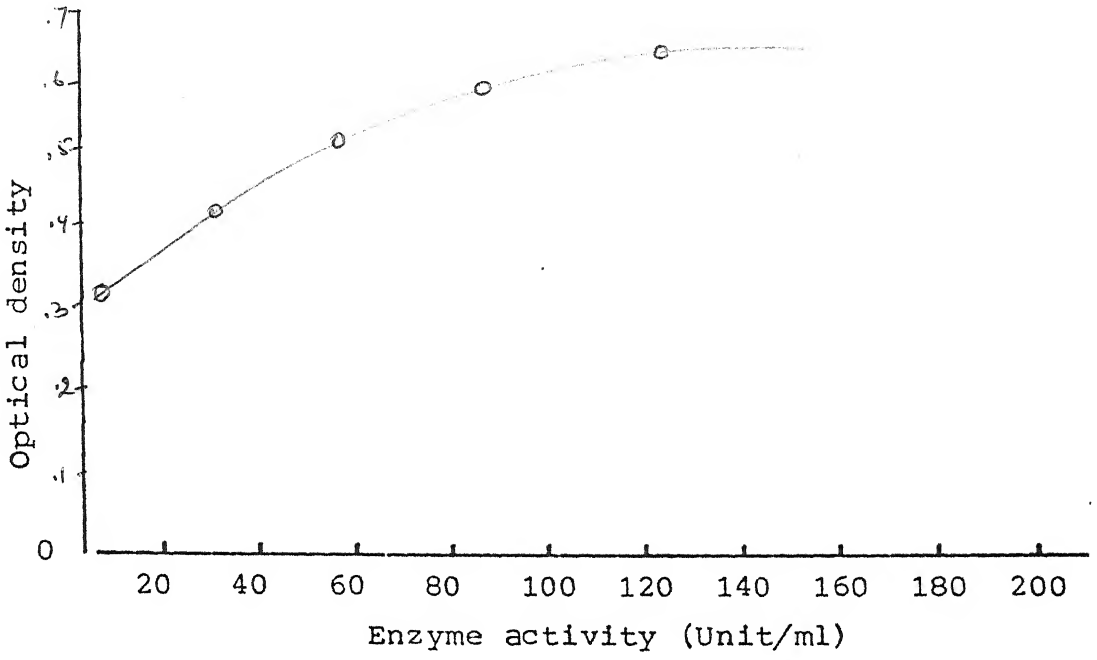
Clean, dry test tubes were taken and labelled as 1,2,3,4, 5 and T. Thes test tubes were arranged and reagents pippetted into each tube was as follows :

Reagents	Test tube number				
	1	2	3	4	5
Enzyme activity(Units/ml)	0	28	57	97	150
Reagent 1 (ml)	0.5	0.45	0.4	0.35	0.3
Reagent4 (ml)	-	0.05	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
Reagent 2 (ml)	0.5	0.5	0.5	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 minutes.					
Solution I	5.0	5.0	5.0	5.0	5.0

Test tubes were mixed well by inversion and allowed to stand at room temperature for 10 minutes. Optical density was measured of all five test tubes against distilled water on colorimeter with a green filter.

A standard graph was plotted by taking enzyme

activity on X axis and optical density on Y axis.



Test Tube :

Reagent 1 : 0.5 ml (incubated at 37°C for 5 minutes)

Serum : 0.1 ml (mixed well and incubated at 37°C for 30 minutes.

Reagent 2 : 0.5 ml (mixed well and allowed to stand at room temperature for 20 minutes.)

Solution I : 5.0 ml.

Test tube was mixed well and stand at room temperature for 10 minutes and read the optical density against distilled water on colorimeter using a green filter.

Calculation

The optical density of test was marked on the Y_axis of the standard curve and extrapolate it to the corresponding enzyme activity on X-axis.

O B S E R V A T I O N S

OBSERVATIONS

The present study "Liver dysfunction in severe birth asphyxia" was carried out from September, 1993 to August, 1994 in the department of Pediatrics, M.L.B. Medical College, Hospital, Jhansi (U.P.).

In the present study 30 severely birth asphyxiated neonates were taken as study group (B) and eleven normal full term neonates served as control group (A). General characteristics are given in table I.

TABLE I : General characteristics of neonates of both the groups.

Characteristics	Control group (n=11)	Study group (n=30)	p value
Sex :			
Male	7 (63.63%)	18(60.0%)	>0.05
Female	4 (36.36%)	12(40.0%)	>0.05
Gestational age(weeks)			
Mean±S.D.	38.90±0.83	39.50±1.04	>0.05
Range	38 to 40	39 to 41	
Weight (gms)			
Mean±S.D.	2,790±181	2,798±206	>0.05
Range	2,500 to 3,000	2,500 to 3,100	

The mean gestational age of neonates of study and control groups was 39.5±1.04 and 38.9±0.83 weeks respectively. While the weight of study and control group was 2798±206 and 2790±181 gms respectively. The difference

between two groups was statistically insignificant ($p > 0.05$) (Table I).

Out of 30 neonates of study group (B), 18 (60%) were males and remaining 12(40%) were females. While in the control group (A), out of 11 neonates, 7(63.63%) were males and rest 4(36.36%) were females. The difference was statistically insignificant.

All the neonates of study group were well matched with neonates of control group in terms of sex, gestational age and weight.

TABLE II : Mean±S.D. and range of liver enzymes in study and control group cases.

Serum enzymes	Study group Mean±S.D. (Range)	Control group Mean±S.D. (Range)	p value
SGOT (IU/L)	112.87±54.70 (36 to 234)	48.73±16.08 (24 to 76)	< 0.001
SGPT (IU/L)	51.17±35.03 (18 to 154)	20.54±6.99 (12 to 30)	< 0.01
Alkaline phosphatase	17.64±2.28 (11.6 to 21.3)	14.45±1.53 (11.8 to 17.0)	< 0.001
Serum bilirubin	6.47±2.61 (3.1 to 12.7)	5.32±2.49 (1.6 to 8.2)	> 0.05

Statistical comparison of study group with control group for liver enzymes.

SGOT : p < 0.001 highly significant
SGPT : p < 0.01 highly significant
Alkaline phosphatase : p < 0.001 highly significant.
Serum bilirubin : p > 0.05 not significant

As evident from table II, in control group, the mean values of SGOT, SGPT and Alkaline phosphatase were 48.73±16.08 IU/L, 20.54±6.99 IU/L and 14.45±1.53 KAU/dl respectively. The corresponding values in study group were 112.87±54.70 IU/L, 51.17±35.03 IU/L and 17.64±2.28 KAU/dl respectively. These values were statistically highly significant.

The mean values of serum bilirubin in study and control groups were 6.47±2.61 mg/dl and 5.32±2.49 mg/dl respectively. The values were statistically insignificant ($p > 0.05$) (Table II).

TABLE III : Outcome of asphyxiated neonates with Apgar score ≤3 or >3 at 5 minute.

Apgar Score at 5 minute	Expired	Survived	Total
≤3	9 (90%)	1 (10%)	10
>3	5 (25%)	15(75%)	20

Out of 30 neonates of study group, 10 neonates still had Apgar score of ≤3 at 5 minutes. Among these neonates, 9(90%) expired and only one neonate (10%) survived. Remaining 20 neonates had Apgar score of more than 3 at 5 minutes. Out of these 20 neonates, 15(75%) neonates survived and rest 5(25%) neonates expired (Table III).

TABLE IV : Outcome of neonates in study group as per liver enzymes values.

Liver enzyme	Expired	Survived	p value
Deranged (n=23)	14(60.87%)	9(39.13%)	<0.001
Normal (n=7)	-	7(100.0%)	-
Total (n=30)	14(46.67%)	16(53.33%)	

It was observed that out of 30 neonates of study group, only 23 neonates had deranged liver enzymes while remaining 7 had normal liver enzymes (Table III). Among 23 neonates who had deranged liver enzymes, only 9(39.13%) neonates survived and remaining 14(60.87%) neonates expired. The values were highly significant statistically ($p < 0.001$). All the 7 neonates having normal values of liver enzymes survived. Hence it is clear from table III that there was 60.87% mortality in neonates of study group having deranged liver enzymes whereas in neonates having normal liver enzymes there was no mortality i.e. all the neonates survived.

As depicted in table V, the mean SGOT, SGPT and Alkaline phosphatase values in non survivors were 157.71$\pm$42.94 IU/L, 72.21$\pm$41.49 IU/L and 18.81$\pm$1.71 KAU/dl respectively. While the corresponding values in survivors were 73.63$\pm$25.82 IU/L, 32.75$\pm$10.25 IU/L and 16.59$\pm$2.27

KAU/dl respectively. The difference for enzymes in two groups was statistically highly significant.

TABLE V : Mean±S.D., range values of liver enzymes studied in survived and nonsurvived neonates in study group.

Liver enzymes	Survivors (n=16) Mean±S.D. (Range)	Nonsurvivors (n=14) Mean±S.D. (Range)	p value
SGOT (IU/L)	73.63±25.82 (36 to 116)	157.71±42.94 (94 to 234)	<0.001
SGPT (IU/L)	32.75±10.25 (18 to 46)	72.21±41.49 (38 to 154)	<0.001
Alkaline phosphatase (KAU/dl)	16.59±2.27 (11.6 to 19.6)	18.81±2.71 (16.6 to 21.3)	<0.01
Serum bilirubin (mg/dl)	6.09±2.67 (3.1 to 10.0)	6.91±2.58 (3.4 to 12.2)	>0.05

Statistical analysis of liver enzymes in nonsurvivors versus survivors neonates.

SGOT : $p < 0.001$ Highly significant

SGPT : $p < 0.001$ highly significant

Alkaline phosphatase : $p < 0.01$ highly significant

Serum bilirubin : $p > 0.05$ Not significant

The values of serum bilirubin among nonsurvivors and survivors were 6.91±2.58 mg/dl and 6.09±2.67 mg/dl respectively. The difference was statistically insignificant($p > 0.05$) (Table V).

TABLE VI : Mean ±S.D., range values of liver enzymes studied in survived neonates of study and control groups.

Liver enzymes	Survivors of study group (n=16) Mean±S.D. (Range)	Control group (n=11) Mean±S.D. (Range)	p value
SGOT (IU/L)	73.63±25.82 (36 to 116)	48.73±16.08 (24 to 76)	>0.05
SGPT (IU/L)	35.75±10.25 (18 to 46)	20.54±6.99 (12 to 30)	<0.01
Alkaline phosphatase (KAU/dl)	16.59±2.27 (11.6 to 19.6)	14.45±1.53 (11.8 to 17.0)	>0.05
Serum bilirubin (mg/dl)	6.09±2.67 (3.1 to 10.0)	5.32±2.49 (1.6 to 8.2)	>0.05

Statistical analysis of survived neonates of study group as compared with controls.

SGOT : $p > 0.05$ not significant

SGPT : $p < 0.01$ highly significant

Alkaline phosphatase : $p > 0.05$ not significant

Serum bilirubin : $p > 0.05$ not significant

The mean values of SGOT, SGPT, Alkaline phosphatase and serum bilirubin in survived neonates of study group were 73.62±25.82 IU/L, 32.75±10.25 IU/L, 16.59±2.27, KAU/dl and 6.09±2.67 mg/dl respectively whereas the values of these enzymes among controls were 48.73±16.08 IU/L, 20.54±6.99 IU/L, 14.45±1.53 KAU/dl and 5.32±2.49 mg/dl respectively. These values were statistically insignificant except the values of SGPT which were statistically highly significant ($p < 0.01$) (Table VI).

TABLE VII : Mean±S.D., range values of liver enzymes studied in expired neonates of study and control groups.

Liver enzymes	Expired neonates of study group (n=14) Mean±S.D. (Range)	Control group (n=11) Mean±S.D. (Range)	p value
SGOT (IU/L)	157.71±42.94 (94 to 234)	48.73±16.08 (24 to 76)	<0.001
SGPT (IU/L)	72.21±41.49 (38 to 154)	20.54±6.99 (12 to 30)	<0.001
Alkaline phosphatase (KAU/dl)	18.81±2.71 (16.6 to 21.3)	14.45±1.53 (11.8 to 17.0)	<0.001
Serum bilirubin (mg/dl)	6.91±2.58 (3.4 to 12.2)	5.32±2.49 (1.6 to 8.2)	>0.05

Statistical analysis of expired neonates of study group as compared with controls for liver enzymes studied.

SGOT : p <0.001 highly significant

SGPT : p <0.001 highly significant

Alkaline phosphatase : p <0.001 highly significant

Serum bilirubin : p >0.05 not significant

The levels of SGOT, SGPT and Alkaline phosphatase in expired neonates of study group were 157.71±42.94 IU/L, 72.21±41.49 IU/L, and 18.81±1.71 KAU/dl respectively while the values of these enzymes in controls were 48.73±16.08, IU/L, 30.54±6.99 IU/L and 14.45±1.53 KAU/dl respectively. These values were statistically highly significant (p <0.001) (Table VII).

The values of serum bilirubin in expired neonates of study group and their controls were 6.92± 2.59 mg/dl and 5.32±2.49 mg/dl respectively. The values were statistically insignificant ($p > 0.05$) (Table VII).

Thus, it is clear that those neonates of study group who expired had high level of SGOT, SGPT and Alkaline phosphatase values as compared to their controls. ($p < 0.001$).

D I S C U S S I O N

D I S C U S S I O N

Hypoxia, hypercapnia and acidosis represent, at birth, the main symptoms of fetal distress, which manifests clinically in utero as varying fetal heart rate and after delivery as a low Apgar score.

The determination of liver enzymes could offer a simple and rapid laboratory test for establishing the presence of hepatic cellular damage associated with perinatal asphyxia. As a result of change in cellular permeability, hypoxia causes degeneration in tissues, cloudy swelling, vacuolar cytoplasmic degeneration and cellular necrosis (Vogel, 1967) in about 97% of newborn dying from asphyxia.

The effect of asphyxia on the liver and the hepatic functions of the neonate is a relatively unexplored avenue. This study was, therefore, carried out to assess the effect of birth asphyxia on hepatic function of a neonate as also the ultimate outcome of these cases. The study included 30 severely asphyxiated full term neonates. Neonates were considered to have severe birth asphyxia if the Apgar score was ≤ 3 and ≤ 5 at 1 and 5 minutes respectively.

The infants suffering from significant congenital anomaly, intrauterine infections, heart disease, septicemia, significant hepatosplenomegaly, multiple pregnancies and low birth weight babies were not included in the study.

Preterm infants were excluded from the study as they have more immature liver and are more susceptible to respiratory distress syndrome, infections and other complications which may further complicate the picture of asphyxia.

Asphyxia causes most of system disorders including liver. Damage to liver is reflected by the derangement in its enzymes. In newborns with clinical symptoms of asphyxia, serum transaminase activity increase significantly during first 72 hours of life (Zanardo et al, 1985). Hence in the present study liver function tests (SGOT, SGPT, alkaline phosphatase and serum bilirubin) were measured within first 48-72 hours of life.

The gestational age of neonates was calculated by counting the number of weeks from the first day of last menstrual period till the birth of baby and confirmed by physical and neurological developmental scoring system (modified scoring system for assessment of gestational age of newborn (Meharban Singh et al, 1975).

In the present study the mean gestational age of asphyxiated neonate was 39.50±1.04 weeks and mean birth weight was 2798±206 gms whereas in control group it was 2790±181 gms. Both the groups were well matched as regards to birth weight and gestational age.

In this study the mean level of SGOT in asphyxiated neonates was significantly higher as compared to their controls. The mean value was 112.87±54.70 IU/L in asphyxiated neonates as compared to 48.73±16.08 IU/L

in controls. Out of 30 asphyxiated neonates SGOT was raised in only 73.33% of cases. Similar rise in SGOT level was reported by Zanardo et al (1985) in severely asphyxiated neonates within first 72 hours of birth. They observed a rise to a level of 100±68.9 IU/L in asphyxiated neonates as against 52.0±12.9 IU/L in controls. They also observed that levels of SGOT gradually decreased and become comparable to their controls between 5th and 10th day of life. Between 20th and 30th day of life, the mean values of SGOT in asphyxiated neonates and their controls further decreased and did not exceeded 30 IU/L. Saili et al (1980) also showed similar results with a rise of SGOT levels to 97.84±119.2 IU/L in asphyxiated neonates as compared to 54.83±48.86 IU/L in controls(Mean±2S.D.).

SGPT activity was also raised among asphyxiated neonates in this study as compared to their controls. The mean values of SGPT in asphyxiated neonates and their controls were 51.17±35.03 IU/L and 20.54±6.99 IU/L respectively. Out of 30 asphyxiated neonates the SGPT was raised in 70% cases. Zanardo et al (1985) also reported raised levels of SGPT in severely asphyxiate neonates in first 72 hours of life. The mean levels reported in their study were 54.4±54.4 IU/L in severely asphyxiated neonates and 18.0±6.6 IU/L in their controls. Then after, they obtained a fall in SGPT levels in full term asphyxiated neonates but the value remained significantly raised as compared to controls. According to their study, the initial rise was more marked for SGOT as compared to SGPT but SGPT remained significantly raised even upto 30th day

of life. Whereas, SGOT levels returned to normal within 5th to 10th day of life in asphyxiated neonates.

Saili et al (1989) reported similar rise in SGPT values during first 72 hours. The level reported by them was 44.09±61.94 IU/L (Mean±2S.D.) in severely asphyxiated newborns and 22.4±32.96 IU/L in their controls.

In this study the levels of alkaline phosphatase were also raised as compared to their controls. The mean value among asphyxiated neonates was 17.64±2.28 KAU/dl and in their control was 14.45±1.53 KAU/dl. Out of 30 asphyxiated neonates, alkaline phosphatase levels were raised in 63.33% of cases. Similar results were observed by Saili et al (1989). The mean value of alkaline phosphatase in their study was 17.64±12.30 KAU/dl in severely asphyxiated neonates and 14.36±9.06 KAU/dl in their controls (mean±2S.D.). The levels of alkaline phosphatase were raised in 58% of cases in their study. But Fitz Simsons (1984) found no significant increase in the values of alkaline phosphatase in asphyxiated neonates in their study.

The present study also registered increased serum bilirubin in all neonates of both study and control groups. The mean value of serum bilirubin in asphyxiated neonates was 6.47±2.61 mg/dl, while mean value in their controls was 5.32±2.49 mg/dl. Similar results were reported by Saili et al (1989). In their study the mean level of serum bilirubin among severely asphyxiated neonates was 4.78±

6.62 mg/dl and in their controls was 4.50±6.12 mg/dl (Mean±2S.D.).

The mean levels of transaminases are significantly higher among normal healthy full term neonates as compared to older children and adults. The adult levels of transaminases is achieved by the end of neonatal period. The higher transaminase activity in neonatal blood has been attributed to seepage from the hepatocytes of neonates, which, being immature, have more permeable membranes. Increased transaminases in newborns may also be the result of increased biosynthesis, physiologic hemolysis of erythrocytes and skeletal muscle trauma during birth(Wolf,1981).

Due to asphyxia the liver may be so damaged ("Shock Liver") that it may not provide its basic functions (Cloherty et al, 1985). In the event of hepatic cellular injury due to perinatal hypoxia, transaminases activity in the blood increases due to changes in cell membrane permeability or cellular necrosis of hepatocytes (King et al, 1959). In the presence of liver cell necrosis, both the cytoplasmic and mitochondrial enzymes are increased. Since SGOT is also present in myocardium, kidneys and RBCs while SGPT is primarily released from the liver, therefore, this enzyme is more specific for liver damage or injury. Alkaline phosphatase also rises in the liver damage but it is less specific and sensitive.

The serum bilirubin values are not taken into account as there is physiological rise even in healthy

neonates due to multifactorial etiology during first few days of life and the difference in study and control group was found to be insignificant.

Asphyxiated neonates were divided into the following two groups :

Group A : Asphyxiated neonates who expired in the hospital.

Group B : Asphyxiated neonates who improved and survived and were discharged from the hospital.

Out of 30 asphyxiated neonates in our study, 14 neonates expired (46.67%) and 16 survived (53.33%). The mean values of liver enzymes of these two groups were calculated and compared (Table V).

Similar results were observed by Saili et al (1989). Levels reported in their study for SGOT, SGPT and alkaline phosphatase among neonates who expired, were 146.2±102.4 IU/L, 61.1±51.52 IU/L and 20.3±12.2 KAU/dl respectively. Whereas levels of these enzymes among survivors of asphyxiated neonates in their study were 62.9±73.6, 31.7±58.0 IU/L and 15.7±11.18 KAU/dl for SGOT, SGPT and Alkaline phophatase respectively.

In this study, SGOT levels were raised in 100% of neonates , SGPT levels were raised in 92.86% of neonates and alkaline phosphatase levels were raised in 70.57% of non survivors from the study group. Whereas serum bilirubin was raised in 100% of cases. Out of 14, neonates, who expired, only 78.57% of neonates had raised levels of all the three enzymes viz. SGOT, SGPT and

alkaline phosphatase.

Among 16 asphyxiated neonates, who survived, SGOT, SGPT and alkaline phosphatase were deranged in only 50% of cases. Only 37.5% of these cases had all three enzymes viz. SGOT, SGPT and alkaline phosphatase deranged.

No significant difference was observed in the mean values of SGOT, alkaline phosphatase and serum bilirubin levels between controls and asphyxiated neonates who survived. However, SGPT values were significantly raised in asphyxiated neonates who survived as compared to their controls (Table VI).

This study thus conclude that liver damage due to asphyxia is minimal in neonates who survived as compared to neonates who expired in the hospital.

It was interesting to note that asphyxiated neonates having deranged liver enzymes had poor prognosis as compared to neonates having normal levels of liver enzymes (Table IV). Out of 23 neonates who showed deranged liver enzymes, 60.87% expired in contrast to neonates having normal liver enzymes, all of whom survived. Saili et al (1989) recorded deranged liver enzymes in 60% of cases, who expired due to asphyxia.

On further analysis, it was found that enzyme derangement was present in 23 (76.67%) neonates, Out of 30 asphyxiated neonates. All the 14 non-survivors felt in this group. Rest of neonates (23.33%) had normal liver function tests, none of them expired.

It could be concluded from our observations that when all the enzymes are deranged in an asphyxiated neonate a gaurded prognosis have to be explained to the relatives.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study was carried out to study the "Liver dysfunction in severe birth asphyxia". The cases were studied from September, 1993 to August, 1994 in the Department of Paediatrics, M.L.B. Medical College, Hospital, Jhansi (U.P.).

The study group comprised of 30 severely birth asphyxiated full term neonates. Diagnosis of severe birth asphyxia was made when Apgar score was ≤ 3 and ≤ 5 at 1 and 5 minutes respectively. Eleven full term normal neonates were taken as controls. Neonates who had significant problems viz. significant congenital anomalies, septicemia, history of leaking more than 12 hours or absent membrane, heart disease, renal failure, significant hepatosplenomegaly, preterm neonates, twins and low birth weight neonates were excluded both from study and control groups.

Liver enzymes viz. SGOT, SGPT, alkaline phosphatase and serum bilirubin were estimated in serum of neonates included in this study. Blood was collected from peripheral vein with all aseptic precautions within first 48 to 72 hours of life.

The assessment of gestational age was done by recording the first day of last menstrual period and confirmed by physical and neurological developmental score (modified scoring system for assessment of gestational age in newborn by Meharban Singh et al, 1975).

In the present study, the mean gestational age was 38.90±0.83 weeks in control group and 39.56±1.04 weeks in neonates of study group. In study group 60% neonates were male and 40% were females. The mean birth weight of study group was 2,798±206 gms and 2,790±181 gms in controls. The study group neonates were well matched with their control group neonates in terms of gestational age, sex and birth weight.

SGOT, SGPT and alkaline phosphatase values are significantly higher in neonates of study group (112.87± 54.70 IU/L, 51.17±35.03 IU/L and 17.64±2.28 KAU/dl for SGOT, SGPT and alkaline phosphatase respectively) as compared to the neonates of control group (48.73±16.08 IU/L, 20.54±6.99 IU/L and 14.45±1.53 KAU/dl for SGOT, SGPT and Alkaline phosphatase respectively).

Among asphyxiated neonates (study group) SGOT, SGPT and alkaline phosphatase were significantly raised who succumbed to asphyxia (157.71±42.94 IU/L, 72.21± 41.43 IU/L and 18.81_+2.71 KAU/dl for SGOT, SGPT and Alkaline phosphatase respectively) than those who survived (73.63±25.82 IU/L, 32.75±10.25 IU/L and 16.5± 2.27 KAU/dl for SGOT, SGPT AND ALKALINE PHOSPHATSE respectively. The levels of serum bilirubin were also raised who expired (6.91±2.58 mg/dl) than those who survived (6.09±2.67 mg/dl) but the difference was statistically insignificant.

Out of 30 neonates of study group, 23 had

deranged liver enzymes while rest 7 had normal oiver enzymes. Among 23 neonates who had deranged liver enzymes, 14 expired (60.87%) and remaining 9(39.13%) survived. All the 7 neonates of study group who had normal liver enzymes survived.

In asphyxiated group, those neonates who still had Apgar score of ≤ 3 at 5 minutes had significantly higher values for SGOT, SGPT and alkaline phosphatase and 90% of them expired than those who had Apgar score of >3 at 5 minutes and only 25% of them expired.

CONCLUSION

Severe birth asphyxia causes liver dysfunction as evident by deranged SGOT, SGPT and alkaline phosphatase. Deranged liver enzymes could be detected in first 48 hours of life. Approximately 76.66% of severely asphyxiated neonates suffered from liver derangement. Enzyme derangement is significantly more in those neonates who succumbed to asphyxia than those who survived. SGPT levels were significantly deranged than SGOT levels among asphyxiated neonates as a whole.

In asphyxiated group, those neonates who had Apgar score of ≤ 3 at 5 minutes had significant higher values of SGOT, SGPT and Alkaline phosphatase and 90% of them expired than those who had Apgar score of >3 at 5 minutes.

B I B L I O G R A P H Y

B I B L I O G R A P H Y

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A P P E N D I X

APPENDIX

WORKING PROFORMA

TOPIC - LIVER DYSFUNCTIONS IN SEVERE BIRTH ASPHYXIA

Mother's Name :

Case No.

Age of Baby :

MRD No.

Sex

D.O.A.

ANTENATAL HISTORY

Pre-eclampsia
Eclampsia
Septicemia
Diabetes
APH
Convulsions

NATAL HISTORY

Presentation :

Mode of delivery : Vaginal/Forcep/Caessarean

History of any Medication : given during delivery

POSTNATAL HISTORY :

Any Medication used :

Apgar scoring :

	1 minute	5 minute
Colour		
Heart rate		
Respiratory rate		
Response to Stimuli		
Muscle tone		

EXAMINATION OF BABY

Assessment of Gestational Age

1. Last Menstrual Period
2. Morphological Examination
3. Neurological Examination

Anthropometric Examination

Weight	kg.	Head Circumference	cm
Height	cm.	Chest circumference	cm

General Examination

General appearance
Colour
Cry
Activity
Posture

Any congenital anamoly
Head ---- Caput/ Cephal Haematoma
Face
Neck
Skin ---- Icterus

Systemic Examination

Cardiovascular System :

Respiratory System :

Abdominal Examination :

Central Nervous System :

Moro's reflex	Glabellar Tap
Rooting	Sucking
Swallowing	Palmar grasp
Plantar	

INVESTIGATION

1. Serum Bilirubin
2. Alkaline Phosphatase
3. S.G.O.T.
4. S.G.P.T.
5. Other investigations whenever needed.

CRITERIA	Score 0	1	2	3
II. NEUROLOGICAL				
a. Posture	Arms and legs extended	Beginning of flexon of hips and knees Arm extended.	Strong flexon of leg and some flexon of arms.	Leg flexed and abducted while arms completely flexed.
b. Arm recoil	No recoil or only random movements.	Arm returns to incompleye flexon or sluggish response.	Arm briskly returns to full flexion.	
c. Poplteal angle	180°	180-150°	150-120°	120-90°
d. Head lag	Complete head lag	Partial head control	Able to maintain head in the line with body	Bring head anterior to body.
e. Glabellar tap	Absent	Weak response	Brisk response.	

Physical Score : 0-6, Neurological Score : 0-13, Combined Total Score : 0-29.

Relationship between combined total score with gestational age.

Gestation(weeks)	Combined total score	Gestation(weeks)	Combined total score
28	9	35	18
29	10	36	19
30	11	37	20
31	12	38	23
32	13	39	25
33	15	40	26
34	16		
